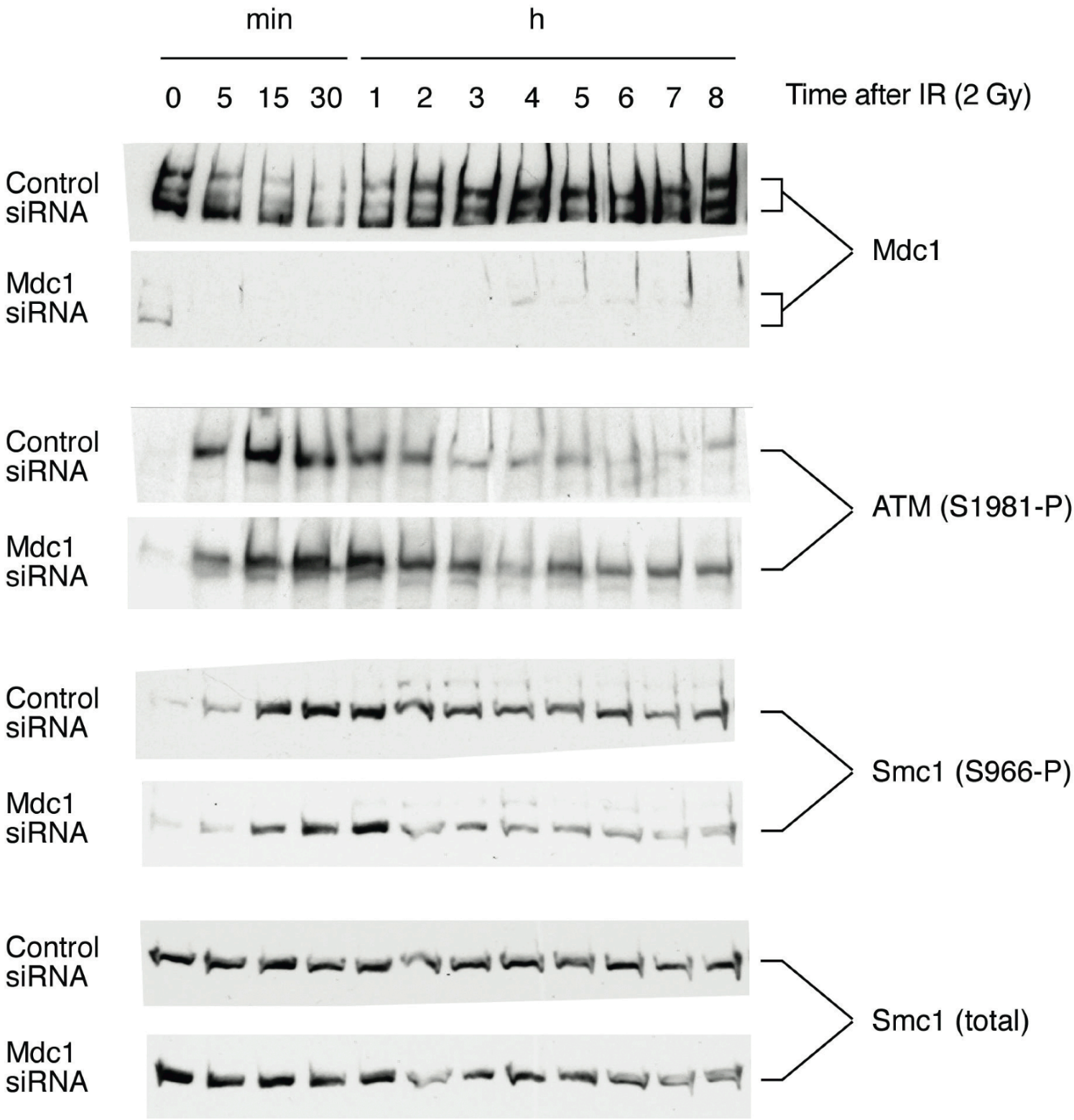
min
h
0 5 15 30 1 2 3 4 5 6 7 8
Time after IR (2 Gy)
Control siRNA
Mdc1 siRNA
Mdc1
Control siRNA
Mdc1 siRNA
ATM (S1981-P)
Control siRNA
Mdc1 siRNA
Smc1 (S966-P)
Control siRNA
Mdc1 siRNA
Smc1 (total)

Legend to Supplementary information 5

Mdc1 is not required for checkpoint initiation but helps modulate the timing and extent of ATM-dependent phosphorylation events.

U-2-OS cells were transiently transfected with control or Mdc1-targeting siRNA duplexes as indicated, and exposed to a low (2 Gy) dose of ionizing radiation (IR). At the indicated time-points, the cell lysates were analyzed by immunoblotting with the indicated antibodies. Note the near-complete efficiency in silencing Mdc1 expression under these conditions (upper panels). ATM activation was measured by the extent of its autophosphorylation on S1981; the extent of phosphorylation of its downstream targets was assessed using phosphorylation of Smc1 (S966; the key downstream effector along the ATM-Nbs1-controlled checkpoint signaling). Note that although both ATM and Smc1 become efficiently phosphorylated in response to this dose of IR, the peaks of their respective phosphorylations are slightly delayed in Mdc1-depleted cells. Total levels of Smc1 serve as loading controls.